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Response To Formal Matters

Applicants express appreciation for the return of the initialed Form PTO-1449, whereby the Examiner's consideration of Applicants' Information Disclosure Statement filed October 9, 2001 and Resubmission of Documents filed August 7, 2002 is of record.

Applicants submit herewith a copy of U.S. Patent No. 6,436,912 B1 which issued on August 20, 2002 accompanied by a Form PTO-1449. Applicants note that the family member WO 97/46420 and U.S. Application No. 09/147,342 were cited in the Information Disclosure Statement filed October 9, 2001, and this document is being submitted to update the record. Accordingly, no fee should be necessary for consideration of this document. However, if any fees are necessary, including any fee under 37 C.F.R. 1.17(p), authorization is given herein to charge any necessary fee to Deposit Account No. 19-0089. Applicants respectfully request that an initialed copy of the Form PTO-1449 be forwarded to Applicants with the next communication from the Patent and Trademark Office.

Applicants further express appreciation for the indication that the drawings filed with the application on July 2, 2001 are accepted.

Still further Applicants express appreciation for the acknowledge of the claim of priority and receipt of the certified copies in this national stage application.

The abstract and claims have been amended to indicate that "DDS" is "drug delivery system" in accordance with page 1 of Applicants' specification, in the first line under "Technical Field".

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Applicants provide the inventors' residences as follows:

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Applicants respectfully submit that submission of inventor residences herein should be sufficient to complete the record. However, if a formal Application Data Sheet repeating all the submitted information is needed, the Examiner is respectfully requested to contact the undersigned.

The objections to the specification and claims have been addressed in the amendments herein. In particular, SEQ ID NOS have been inserted in the specification and claims, and typographical errors have been corrected. However, at page 13, line 20, the claim number has not been canceled, because it refers to claim 2 of the Japanese patent document. Accordingly, the objections should be withdrawn.

Response To Restriction Requirement

Applicants note that the Office Action suggests the deletion of the non-elected sequence from the claims in order to expedite prosecution. In this regard, the Office Action indicates that the non-elected sequence will not be rejoined because the election is the result of a restriction between patentably distinct sequences.

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In response, Applicants respectfully submit that the requirement is, in fact, a species requirement, whereby upon allowance of a generic claim rejoinder of a reasonable number of non-elected species is required by the rules, i.e., 37 C.F.R. 1.141. Moreover, the MPEP, 8th Edition, Section 809.02(b), August 2001, page 800-50 indicates that:

(1) when *all* claims to each of the additional species are embraced by an allowable generic claim as provided by 37 CFR 1.141, applicant must be advised of the allowable generic claim and that claims drawn to the nonelected species are no longer withdrawn since they are fully embraced by the allowed generic claim.

Accordingly, Applicants' request rejoinder of the non-elected species upon allowance of a generic claim is in conformance with Patent and Trademark Office rules and procedures, and is respectfully indicated to be appropriate under the present circumstances.

Response To Indication Of Allowable Subject Matter

Applicants express appreciation for the indication that claims 26, 34, 35 and 38 are not rejected over prior art, but are indicated to be allowable if rewritten to be in independent form and to overcome the objection to claims 34 and 38.

In response, the claims have been amended to substantially include the limitations of the base claims and any intervening claims and to address the objection to claims 34 and 38. Accordingly, these claims and the claims dependent therefrom, i.e., claims 27-33, 36, and 37, are in condition for allowance, and an early mailing of the Notices of Allowance and Allowability is respectfully requested.

Response To Rejections

The following rejections appear in the Office Action:

- (a) Claims 24, 25 and 27-30 are rejected under 35 U.S.C. 102(b) as being anticipated by EP 0 712 635.
- (b) Claim 32 is rejected under 35 U.S.C. 103(a) as being obvious over EP 0 172 635.
- (c) Claim 36 is rejected under 35 U.S.C. 103(a) as being obvious over EP 0 712 635, as applied against claim 32, and further in view of JP 6-87746.
- (d) Claims 1-11, 15-18 and 21-23 are rejected under 35 U.S.C. 102(b) as being anticipated by WO 97/46260, with the rejection indicating that EP 0 916 348 is being used as an English translation of the international publication.
- (e) Claims 1-23 are rejected under 35 U.S.C. 103(a) as being obvious over WO 97/46260, as applied against claims 1-11, 15-18 and 21-23, and further in view of JP 6-87746 and Theodore et al. (U.S. Patent No. 5,886,143), Gonsho et al. ("Tissue-Targeting Ability of Saccharide-Poly(L-Lysine) Conjugates", Biol. Pharm. Bull., Vol. 17, No. 2, pp. 275-282 (1994)), Hashida et al. (Targeted Delivery of Drugs and Proteins to the Liver Via Receptor-Mediated Endocytosis. Journal of Controlled Release, 1997, Vol. 46, pp. 129-137), Kichler et al. (Versatile Synthesis of Bi- and Tri-Antennary Galactose Ligands, Glycoconjugate Journal, 1995, Vol. 12, pp. 275-281) or NISHIKAWA et al. ("Synthesis and Pharmacokinetics of a New Liver-Specific Carrier, Glycosylated Carboxymethyl-Dextran, and Its Application to Drug Targeting", Pharmaceutical Research, Vol. 10, No. 9, pp. 1253-1261 (1993)).

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- (f) Claims 24, 27-33, 36 and 37 are rejected under 35 U.S.C. 103(a) as being obvious over WO 97/46260, as applied against claims 1-11, 15-18 and 21-23, and further in view of EP 0 712 635.

In response, Applicants initially note that claims 26-38 should be in condition for allowance, because they include the subject matter indicated to be allowable by the Examiner. In this regard, Applicants respectfully submit that the reasons for allowance of these claims are not restricted to the reasons cited by the Examiner. The prior art of record in the present application does not collectively disclose the combination of inventive features recited in each of the claims of the present application. Accordingly, Applicant respectfully submits, for the sole purpose of completing the record, that the reasons for allowance of the present application are not limited to the Examiner's comments.

Regarding claims 1-20, 22 and 23, Applicants note that the independent claims have been amended to include galactose or galactosamine. In particular, independent claim 1 is directed to a drug delivery system compound which comprises a carboxy(C₁₋₄)alkyldextran polyalcohol modified with galactose or galactosamine and a residue of drug compound bound to the carboxy(C₁₋₄)alkyldextran polyalcohol. Independent claim 6 is directed to a drug delivery system compound which is obtainable by binding a residue of a drug compound to a carboxy(C₁₋₄)alkyldextran polyalcohol in which a part of carboxyl groups of the carboxy(C₁₋₄)alkyl moiety are modified with galactose or galactosamine. Independent claim 9 is directed to a drug delivery system compound which is obtainable by modifying with a galactose or galactosamine a carboxy(C₁₋₄)alkyldextran polyalcohol in which a residue of a drug compound is bound to a part of carboxyl groups of the carboxy(C₁₋₄)alkyl moiety by a spacer. Independent claim 22 is directed to a carboxy(C₁₋₄)alkyldextran polyalcohol modified with galactose or galactosamine. Independent claim 23 is

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directed to a polymer carrier comprising a carboxy(C₁₋₄)alkyldextran polyalcohol modified with galactose or galactosamine.

With regard to the above, Applicants note that carboxy(C₁₋₄)alkyldextran polyalcohol achieves an extremely prolonged serum retention, i.e., prolonged high concentration in serum, as compared to carriers disclosed in the references, but attributable to this property, the carrier provides a DDS compound which can hardly penetrate or distribute into tissues or organs. The DDS compound of the present invention solves this problem by modification by galactose or galactosamine.

Expanding upon the above, attention is directed to Applicants' Example 6 in the specification, wherein the DDS compound of the present invention accumulates at a higher concentration in the liver than a DDS compound with galactose modification. See, for example, Applicants' specification, at page 4, the paragraph beginning at the bottom of the page, page 18, lines 14-8, page 22, first full paragraph, and Example 6. The same DDS compounds with or without galactose modification were compared in serum concentration and urinary concentration. As a result, these compounds were revealed to give almost the same pharmacokinetic properties, as seen in the attached figures for serum and urine. These results in combination with the results shown in Example 6 suggest that the presently claimed DDS compounds accumulate selectively in the liver and give an unexpectedly high concentration in spite of the presence of carboxy(C₁₋₄)alkyldextran polyalcohol, while the compound presumably gives very limited distribution in the other organs or tissues due to the high serum retention provided by carboxy(C₁₋₄)alkyldextran polyalcohol as in the same manner as the DDS compound without galactose modification.

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If the Examiner would like any further information regarding the figures, the Examiner is requested to contact the undersigned.

Applicants respectfully submit that any combination of the prior art of record does not teach or suggest Applicants' disclosed and claimed invention. In particular, any combination of WO 97/46260, EP 0 916 348, JP 6-87746 and Theodore et al., Gonsho et al., Hashida et al., Kichler et al., Nishikawa et al. and EP 0 712 635.

Thus, Applicants respectfully submit that Applicants' claims patentably define their invention, whereby withdrawal of the rejections of record is respectfully requested.

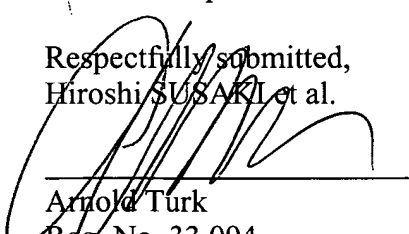
CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the objections and rejections of record, and allow all the pending claims.

Allowance of the application is requested, with an early mailing of the Notices of Allowance and Allowability.

If the Examiner has any questions or wish to further discuss this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,
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APPENDIX
MARKED UP COPY OF AMENDMENTS TO SPECIFICATION

Marked up copy of amended paragraph appearing at page 7, line 20 to page 8, line 7:

According to further preferred embodiments of the aforementioned method of the present invention, there are provided the above method wherein the polymer carrier is those having carboxyl groups, preferably a polysaccharide derivative having carboxyl groups; the above method wherein the polymer carrier is a carboxy(C₁₋₄)alkyldextran polyalcohol, preferably carboxymethyldextran polyalcohol; the above method wherein the dextran polyalcohol that constitutes the carboxy(C₁₋₄)alkyldextran polyalcohol is a dextran polyalcohol which is obtained by treating a dextran under conditions that enable substantially complete polyalcoholization; the above method wherein the polymer carrier is modified with a saccharide compound; the above method wherein the drug compound introduced to the DDS compound is an antineoplastic agent or an anti-inflammatory agent; the above method wherein the spacer is a tetrapeptide represented as, from the N-terminal, -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) or a tetrapeptide represented as, from the N-terminal, -Gly-Gly-Gly-Phe- (SEQ ID NO. 8); the above method wherein the spacer is a group represented as, from the N-terminal, -Gly-Gly-Phe-Gly-NH-Y'-CH₂-O-CO- (SEQ ID NO. 1) or -Gly-Gly-Gly-Phe-NH-Y'-CH₂-O-CO- (SEQ ID NO. 8) wherein Y' represents p-phenylene group; the above method wherein the peptidase is α -chymotrypsin or papain; and the above method wherein the drug compound is (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione.

Marked up copy of amended paragraph appearing at page 8, lines 8 to 19:

According to a particularly preferred embodiment of the above method of the present invention, the above method can be used for measurement of a DDS compound in which a carboxy(C₁₋₄)alkyldextran polyalcohol and (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione are bound to each other by means of a spacer comprising a tetrapeptide represented as, from the N-terminal, -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) or a tetrapeptide represented as, from the N-terminal, -Gly-Gly-Gly-Phe- (SEQ ID NO. 8), and the DDS compound or a content of the antineoplastic agent introduced to the DDS compound can be measured by using α -chymotrypsin as the peptidase, and by measuring (1S,9S)-9-ethyl-5-fluoro-1-glycylamino-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo-[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione as the hydrolysate.

Marked up copy of amended paragraph appearing at page 13, line 26 to page 14, line 7:

Specific examples of oligopeptides that can be used as the spacer are shown in the following table; however, spacers used for the DDS compounds of the present invention are not limited to those mentioned below. It can be readily understood that one of ordinary skilled in the art can appropriately determine whether or not a spacer is used, or choose the type of a spacer when a spacer is used so as to achieve an optimum releasing rate of a drug compound. In the table, the left ends of peptide sequences are N-terminals and the residues of drug compounds are bound to C-terminals. D-Phe represents the D-phenylalanine residue and the other amino acids represent L-amino acids. The degrees of the releasing rate were judged from the degree of

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appearance of efficacy of the DDS compounds carrying doxorubicin against Walker 256 tumor-bearing rats, or from free doxorubicin concentrations at tumorous sites of Walker 256 tumor-bearing rats. For doxorubicin, a spacer that can release the drug compound at a high concentration immediately, e.g., -Gly-Gly-Phe-Gly- (SEQ ID NO. 1), is preferably used among the listed spacers.

Marked up copy of amended paragraph appearing at page 21, line 30 to page 22:

The DDS compound of the present invention is characterized in that it can specifically exhibit desired pharmacological activity at a local site such as tumorous sites or inflammatory sites depending on the sort of a residue of a drug compound (e.g., residues of drug compounds such as antineoplastic agents or anti-inflammatory agents), and can reduce toxicity inherent to the drug compound, per se. Furthermore, the DDS compound of the present invention also has excellent blood vessel permeability. Since [s] protease (peptidase) is expressed at tumorous sites or inflammatory sites, the DDS compound having a spacer comprising an oligopeptide is readily hydrolyzed at the spacer moiety to allow the released drug compound to be incorporated into cells and exhibit its efficacy, or the DDS compound is taken into the cells with the aid of a receptor present in a target cell which recognizes the saccharide, and the drug compound released by the action of a protease exhibits its efficacy.

Marked up copy of amended paragraph appearing at page 27, penultimate line to page 28,
line 12:

A DDS compound (Compound 1) in which a carboxymethyldextran polyalcohol (occasionally abbreviated as “CM-Dex-PA” or “CM-dextran polyalcohol” hereinafter in the examples) as a polymer carrier and an antineoplastic agent ((1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione disclosed in claim 2 of Japanese Patent Unexamined Publication (KOKAI) (Hei) No. 6-87746/1994 (abbreviated as “DX-8951” in hereinafter in the examples) were bound by means of a tetrapeptide spacer represented as -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) (the oligopeptide is shown as the sequence from their N-terminals, and others are shown in the same manner hereinafter in the examples) was produced according to the method described in Example 15 of International Publication WO97/46260. As the CM-Dex-PA, that having an average molecular weight of 228K, and a carboxymethylation degree (degree of substitution with carboxymethyl groups per constitutional saccharide residue) of 0.4 was used.

Marked up copy of amended paragraph appearing at page 29, lines 1 to 8:

A DDS compound (Compound 2) in which CM-Dex-PA and DX-8951 were bound by means of a spacer represented by -Gly-Gly-Gly-Phe-NH-(CH₂)₄-CO- (SEQ ID NO.8) was prepared as follows. 5-Aminopentanoic acid (1.0 g), p-toluenesulfonic acid (1.95 g), and benzyl alcohol (5 ml) were allowed to react in toluene (50 ml) at 140°C for 5 hours while removing the produced water by using a Dean-Stark apparatus. The reaction mixture was concentrated, and the resulting residue was solidified by adding ether. The solid obtained was filtrated, washed with ether, and dried to obtain 2.9 g of tosylic acid salt of 5-aminopentanoic acid benzyl ester.

Marked up copy of amended paragraph appearing at page 29, line 9 to page 30, line 6:

Boc-Gly-Gly-Gly-Phe-OH (575 mg) (SEQ ID NO. 8), HOSu (182 mg), and DCC (326 mg) were dissolved in DMF (20 ml), and the mixture was stirred for 30 minutes. The solution was added with a solution of p-toluenesulfonic acid salt of 5-aminopentanoic acid benzyl ester (500 mg) and triethylamine (0.184 ml) dissolved in DMF (10 ml), and the mixture was stirred for 3 days at room temperature. The reaction mixture was concentrated, and the residue was purified by column chromatography (CH₂Cl₂:MeOH = 20:1) to obtain 380 mg of Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-COOBzl (Bzl represents benzyl group) (SEQ ID NO. 8). The Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-COOBzl (SEQ ID NO. 8) (380 mg) was dissolved in methanol containing 50% of water (20 ml), and the solution was added with 5% Pd-C (water content; 50%, 300 mg) and stirred overnight under hydrogen at ordinary pressure. The catalyst in the reaction mixture was removed by filtration, and the filtrate was concentrated to dryness to obtain Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-COOH (SEQ ID NO. 8) (330 mg).

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Marked up copy of amended paragraph appearing at page 30, lines 7 to 23:

The Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-COOH (SEQ ID NO. 8) (150 mg), DCC (70 mg) and HOSu (40 mg) were dissolved in DMF, and the solution was stirred for 30 minutes. A solution of DX-8951 (160 mg) and triethylamine (0.040 ml) dissolved in DMF was added to the above solution, and then the mixture was stirred overnight at room temperature. The reaction mixture was concentrated, and the resulting residue was purified by column chromatography (CH₂Cl₂:MeOH = 20:1) to obtain Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 8) (110 mg). The Boc-Gly-Gly-Gly-Phe-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 8) (110 mg) was dissolved in TFA (2 ml), and the solution was allowed to react for 1 hour. The reaction mixture was concentrated, and the resulting residue was solidified by addition of ether. The supernatant was removed, and the solid was dried to obtain 100 mg of trifluoroacetic acid salt of H-Gly-Gly-Gly-Phe-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 8).

¹H-NMR (DMSO-d₆): δ 8.45-8.55 (m, 2H), 8.28-8.35 (m, 2H), 7.95-8.10 (br, 2H), 7.79 (d, 1H, J=10.7Hz), 7.70-7.75 (m, 1H), 7.32 (s, 1H), 7.20-7.30 (m, 5H), 7.15-7.25 (m, 4H), 6.50-6.60 (br, 1H), 5.50-5.60 (m, 1H), 5.40-5.50 (m, 2H), 5.18 (s, 2H), 4.50-4.60 (m, 1H), 3.55-3.95 (m, 7H), 3.00-3.25 (m, 5H), 2.75-2.85 (m, 1H), 2.50 (s, 3H), 2.15-2.25 (m, 4H), 1.86-2.00 (m, 2H), 1.55-1.65 (m, 2H), 1.45-1.55 (m, 2H), 0.88 (t, 3H, J=7.35Hz)

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Marked up copy of amended paragraph appearing at page 30, line 25 to page 31, line 11:

CM-Dex-PA (350 mg) produced by the method described in Example 13 of WO 97/46260, having an average molecular weight of 337K and a carboxymethylation degree (degree of substitution with carboxymethyl groups per constitutional saccharide residue) of 0.4, was dissolved in water (10 ml). To this solution, a solution of trifluoroacetic acid salt of H-Gly-Gly-Gly-Phe-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 8) (50 mg) dissolved in methanol (10 ml) was added, and the mixture was further added with a solution of HOBt (7 mg) dissolved in methanol (5 ml). The reaction mixture was adjusted to pH 7.0, added with water-soluble carbodiimide (10 mg), and then the mixture was stirred for 14 hours. The reaction mixture was further added with water-soluble carbodiimide (10 mg), stirred for 2 hours, and then added with water-soluble carbodiimide (10 mg) and stirred for 2 hours. The reaction mixture was diluted with ultrapure water, and the low molecular weight substances were removed by using an ultrafiltration membrane (50K). The filtrate was lyophilized, and the resulting powder was dissolved in 3 M aqueous NaCl, and the solution was added dropwise to ethanol. The deposited solid was separated by centrifugation. After the supernatant was removed, the solid was dissolved in water again. The low molecular weight substances were removed with an ultrafiltration membrane (50K), and the filtrate was passed through a 0.22 µm filter, and lyophilized to obtain 280 mg of the target compound.

Marked up copy of amended paragraph appearing at page 38, line 1:

(C) Synthesis of galactose-modified CM-dextran polyalcohol-Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1)

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Marked up copy of amended paragraph appearing at page 38, line 2 to the end of page 30:

The sodium salt (1.0 g) of the galactose-modified CM-dextran polyalcohol obtained in the above (B) was dissolved in water (30 ml), and the solution was added with a solution of trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (150 mg) (SEQ ID NO. 1) and 1-hydroxybenzotriazole (35 mg) in methanol (40 ml). The solution was adjusted to pH 7.0, and then added with water-soluble carbodiimide hydrochloride (35 mg) 3 times every 2 hours and stirred overnight. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (20 ml), and the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm, 8 minutes). The precipitates were dissolved in water and desalted by ultrafiltration using a Biomax-3 membrane. The residual solution, which did not pass through the membrane, was filtered through a Millipore filter (0.22 μ m), and lyophilized to obtain 900 mg of the title compound. The resulting product was dissolved in 0.1 M aqueous sodium chloride, and analyzed by GPC (column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M aqueous NaCl, flow rate; 0.8 ml/min). The results of the GPC analysis and an ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the compound are shown in Figs. 3 and 4, respectively. The DX-8951 content in the compound was found as 4.9% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

Marked up copy of amended paragraph appearing at page 39, line 1:

(D) Synthesis of CM-dextran polyalcohol-Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1)

Marked up copy of amended paragraph appearing at page 39, line 2 to page 40, line 1:

The sodium salt of the CM-dextran polyalcohol obtained in the above (B) (2.0 g) was dissolved in water, and the solution was passed through Dowex-50 WX8 (Et_3NH^+) to obtain triethylammonium salt of CM-dextran polyalcohol (1.9 g). The resulting triethylammonium salt of CM-dextran polyalcohol (1.9 g) was dissolved in an aqueous solution containing 50% of N,N-dimethylformamide. The solution was successively added with a solution of triethylamine (0.112 ml) and trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (350 mg) in N,N-dimethylformamide (10 ml), and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroxyquinoline (1.9 g), and the mixture was allowed to react overnight at room temperature with stirring. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (20 ml), and the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm). These precipitates were dissolved in water, and desalted by ultrafiltration using a Biomax-3 membrane. The residual solution that did not pass through the membrane was filtered by a Millipore filter (0.22 μm), and lyophilized to obtain 1.4 g of the title compound. The resulting product was dissolved in 0.1 M aqueous sodium chloride, and analyzed by GPC (column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M aqueous NaCl, flow rate; 0.8 ml/min). The result of the GPC analysis and ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the compound are shown in Figs. 6 and 9, respectively. The DX-8951 content in the compound was found as 5.2% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

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Marked up copy of amended paragraph appearing at page 39, line 2 to page 40, line 1:

The resulting sodium salt of galactose-modified CM-dextran polyalcohol (200 mg) was dissolved in water (3 ml), and the solution was added with a solution of trifluoroacetic acid of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (27 mg) in methanol (3 ml) and a solution of 1-hydroxybenzotriazole (7 mg) in methanol (3 ml). The resulting solution was adjusted to pH 7.0, added with water-soluble carbodiimide hydrochloride (7 mg) 3 times every 2 hours, and stirred overnight. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (10 ml), and then the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm). The precipitates were dissolved in water, and desalted by ultrafiltration using a Biomax-50 membrane. The residual solution that did not pass through the membrane was filtered by a Millipore filter (0.22 μ m), and lyophilized to obtain 180 mg of the title compound. The product was dissolved in 0.1 M aqueous sodium chloride, and analyzed by GPC (column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M NaCl aqueous solution, flow rate; 0.8 ml/min). The result of the GPC analysis and an ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the product are shown in Figs. 7 and 10, respectively. The DX-8951 content in the product was and found as 3.7% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

Marked up copy of amended paragraph appearing at page 44, lines 1 to 2:

Example 9: Synthesis of N-acetylgalactosamine-modified CM-dextran polyalcohol-Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1)

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Marked up copy of amended paragraph appearing at page 44, line 15 to page 45, line 12:

The resulting N-acetylgalactosamine-modified CM-dextran polyalcohol (200 mg) was dissolved in water (10 ml), and the solution was added with a solution of trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (30 mg) dissolved in methanol (10 ml), and a solution of 1-hydroxybenzotriazole (30 mg) dissolved in methanol (10 ml). The solution was adjusted to pH 7.0, and added with water-soluble carbodiimide hydrochloride (10 mg) 3 times every 2 hours. The mixture was stirred for 2 hours, and adjusted to pH 8.5. Low molecular weight fractions in the reaction mixture was removed by ultrafiltration using a Biomax -50 membrane. The residual solution that did not pass through the membrane was filtered through a Millipore filter (0.22 μ m) and lyophilized to obtain the title compound (203 mg). The resulting product was dissolved in 0.1 M aqueous sodium chloride and then analyzed by GPC (column; TOSOH TSK Gel PW-6000XL, solvent; 0.1 M acetate buffer (pH 5.0) containing 20% of acetonitrile, flow rate; 0.8 ml/min). The result of the GPC analysis and an ultraviolet absorption spectrum of this compound (0.1 M Tris buffer (pH 10.0):acetonitrile = 7:3, 0.16 mg/ml) are shown in Figs. 8 and 11, respectively. The content of drug compound residue in the product was found as 4.6% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer (pH 10.0):acetonitrile = 7:3.

Marked up copy of amended paragraph appearing at page 45, lines 13 to 14:

Example 10: Measurement of DX-8951 content in CM-Dex-PA-Gly-Gly-Phe-Gly-NH-Y'-CH₂-O-CO-DX-8951 (SEQ IS NO. 1)

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Marked up copy of amended paragraph appearing at page 45, lines 15 to 29:

5 μ l of a solution of CM-Dex-PA-Gly-Gly-Phe-Gly-NH-Y'-CH₂-O-CO-DX-8951 (SEQ ID NO. 1) (Y' means p-phenylene group) prepared as 1 mg/ml in distilled water was added with 95 μ l of a papain solution prepared as 2 mg/ml in Britton Robinson buffer (pH 6). The reaction mixture was incubated at 40°C for 4 hours, added with 100 μ l of 0.5 N HCl solution containing 50% of acetonitrile, and content of the released hydrolysate [DX-8951] was determined by HPLC. For the HPLC analysis, a Symmetry C18 (4.6 x 100 mm; 3.5 μ m, Waters Co.) column was used, and elution was performed with a 0.1% trifluoroacetic acid solution supplemented with an organic solvent (methanol:acetonitrile = 1:2) so as to be a gradient from 20 to 70% for 12 minutes, and the hydrolysate was detected by fluorescent spectroscopy (Ex. 375 nm and Em. 445 nm). As the result, DX-8951 was eluted at about 5.7 minutes. The DX-8951 content in the above DDS compound was calculated as 4.0% by using a calibration curve prepared with DX-8951. On the other hand, the DX-8951 content was calculated as 3.3% based on UV absorption of the aforementioned DDS compound by using a calibration curve prepared with DX-8951.

Marked up copy of amended paragraph appearing at page 45, last line to page 46, line 1:

Example 11: Measurement of DX-8951 content in CM-Dex-PA-Gly-Gly-Gly-Phe-NH-Y'-CH₂-O-CO-DX-8951 (SEQ ID NO. 8)

Marked up copy of amended paragraph appearing at page 46, lines 2 to 16:

5 μ l of a solution of CM-Dex-PA-Gly-Gly-Gly-Phe-NH-Y'-CH₂-O-CO-DX-8951 (SEQ ID NO. 8) prepared as 1 mg/ml in distilled water was added with 95 μ l of a solution of α -

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chymotrypsin prepared as 2 mg/ml in Britton Robinson buffer (pH 6). The reaction mixture was incubated at 40°C for 4 hours and then added with 100 µl of 0.5 N HCl solution containing 50% of acetonitrile, and the content of the released hydrolysate [DX-8951] was determined by HPLC. For the HPLC analysis, a Symmetry C18 (4.6 x 100 mm; 3.5 µm, Waters Co.) column was used, and elution was performed with a 0.1% trifluoroacetic acid solution supplemented with an organic solvent (methanol:acetonitrile = 1:2) so as to be a gradient from 20 to 70% for 12 minutes, and the hydrolysate was detected by fluorescent spectroscopy (Ex. 375 nm and Em. 445 nm). As a result, DX-8951 was eluted at about 5.7 minutes. The DX-8951 content in the above DDS compound was calculated as 2.5% by using a calibration curve prepared with DX-8951. On the other hand, the DX-8951 content was calculated as 1.7% based on UV absorption of the aforementioned DDS compound by using a calibration curve prepared with DX-8951.

Marked up copy of amended paragraph appearing at page 46, lines 17 to 18:

Example 12: Measurement of DX-8951 content in CM-Dex-PA-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1)

Marked up copy of amended paragraph appearing at page 46, line 19 to page 47, line 2:

5 µl of a solution of CM-Dex-PA-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1) prepared as 100 µg/ml in distilled water was added with 95 µl of a papain solution prepared as 2 mg/ml in Britton Robinson buffer (pH 6). The reaction mixture was incubated at 40°C for 4 hours and then added with 100 µl of 0.5 N HCl solution containing 50% of acetonitrile, and the content of the released hydrolysate [NH₂-(CH₂)₄-CO-DX-8951] was

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determined by HPLC. For the HPLC analysis, a Symmetry C18 (4.6 x 100 mm; 3.5 μ m, Waters Co.) column was used, and elution was performed with 0.1% trifluoroacetic acid solution containing 32% of organic solvent (methanol:acetonitrile = 1:2), and the hydrolysate was detected by fluorescent spectroscopy (Ex. 375 nm and Em. 445 nm). As a result, the NH_2 -(CH_2)₄-CO-DX-8951 was eluted at about 5.3 minutes. The DX-8951 content in the above DDS compound was calculated as 3.0% by using a calibration curve prepared with NH_2 -(CH_2)₄-CO-DX-8951. On the other hand, the DX-8951 content was calculated as 3.1% based on UV absorption of the aforementioned DDS compound by using a calibration curve prepared with DX-8951.

Marked up copy of amended paragraph appearing at page 47, lines 3 to 4:

Example 13: Measurement of DXR content in CM-Dex-PA-Gly-Gly-Phe-Gly-DXR (DXR: doxorubicin) (SEQ ID NO. 1)

Marked up copy of amended paragraph appearing at page 47, line 18:

Example 14: Synthesis of CM-dextran polyalcohol-Gly-Gly-Phe-Gly-DXR (SEQ ID NO. 1)

Marked up copy of amended paragraph appearing at page 47, line 19 to page 48 line 3:

Sodium salt of carboxymethyldextran polyalcohol (30 mg) having an average molecular weight of 274K and a carboxymethylation degree (degree of substitution with carboxymethyl groups per constitutional saccharide residue) of 0.4, which was prepared according to the method described in Example 24 of WO97/46260, was dissolved in 0.05 M collidine-HCl buffer (2 ml)

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containing 50% of methanol. The solution was added with a solution of hydrochloride of Gly-Gly-Phe-Gly-DXR (SEQ ID NO. 1) (4 mg) in methanol (400 μ l), which hydrochloride was prepared according to the method described in Example 43 of WO97/46260, and a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.4 mg) in methanol (240 μ l), and stirred for 2 hours. The solution was added with 30 ml of 3 M brine, and desalted by ultrafiltration using a Biomax-50K membrane. The residual solution that did not pass through the membrane was filtered by a Millipore filter (0.22 μ m), and lyophilized to obtain the title compound (25 mg). The content of the drug compound residue in this compound was determined as 4.3% (w/w) by absorption spectrophotometry at 480 nm in PBS (pH 7.4).

Marked up copy of amended paragraph appearing at page 48, line 4:

Example 15: Synthesis of CM-Dex-PA-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1)

Marked up copy of amended paragraph appearing at page 48, lines 5 to 17:

Boc-Gly-Gly-Phe-Gly-OH (SEQ ID NO. 1) (575 mg), HOSu (182 mg), and DCC (326 mg) were dissolved in DMF (20 ml), and the solution was stirred for 30 minutes. The resulting solution was added with a solution of p-toluenesulfonic acid salt of 5-aminopentanoic acid benzyl ester (500 mg) and triethylamine (0.184 ml) dissolved in DMF (10 ml), and the mixture was stirred at room temperature for three days. The reaction mixture was concentrated, and the residue was purified by column chromatography (CH₂Cl₂:MeOH = 20:1) to obtain 560 mg of Boc-Gly-Gly-Phe-Gly-NH-(CH₂)₄-COOBzl (SEQ ID NO. 1). The Boc-Gly-Gly-Phe-Gly-NH-

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(CH₂)₄-COOBzl (SEQ ID NO. 1) (560 mg) was dissolved in methanol (60 ml) containing 50% of water, and the solution was added with 5% Pd-C (water content; 50%, 1.5 g) and stirred overnight under hydrogen at ordinary pressure. After the catalyst was removed from the reaction mixture by filtration, the mixture was concentrated to dryness to obtain 300 mg of Boc-Gly-Gly-Phe-Gly-NH-(CH₂)₄-COOH (SEQ ID NO. 1).

Marked up copy of amended paragraph appearing at page 48, lines 18 to 24:

The Boc-Gly-Gly-Phe-Gly-NH-(CH₂)₄-COOH (SEQ ID NO. 1) (300 mg), DCC (138 mg) and HOSu (77 mg) were dissolved in DMF, and the solution was stirred for 30 minutes. The resulting solution was added with a solution of DX-8951 (317 mg) and triethylamine (0.078 ml) dissolved in DMF, and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the resulting residue was purified by column chromatography (CH₂Cl₂:MeOH = 10:1) to obtain 400 mg of Boc-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1).

Marked up copy of amended paragraph appearing at page 48, line 25 to page 49, line 3:

The Boc-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1) (300 mg) was dissolved in TFA (2 ml), and the solution was allowed to react for one hour, and then the reaction mixture was concentrated. The resulting residue was solidified by addition of ether, and the supernatant was removed. The solid mass was dried to obtain 250 mg of trifluoroacetic acid salt of H-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1).

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¹H-NMR(DMSO-d₆): δ 8.45-8.55 (m, 2H), 8.28-8.35 (m, 2H), 7.95-8.10 (br, 2H), 7.79 (d, 1H, J=10.7Hz), 7.70-7.75 (m, 1H), 7.32 (s, 1H), 7.20-7.30 (m, 5H), 7.15-7.25 (m, 4H), 6.50-6.60 (br, 1H), 5.50-5.60 (m, 1H), 5.40-5.50 (m, 2H), 5.18 (s, 2H), 4.50-4.60 (m, 1H), 3.55-3.95 (m, 7H), 3.00-3.25 (m, 5H), 2.75-2.85 (m, 1H), 2.50 (s, 3H), 2.15-2.25 (m, 4H), 1.86-2.00 (m, 2H), 1.55-1.65 (m, 2H), 1.45-1.55 (m, 2H), 0.88 (t, 3H, J=7.35Hz)

Marked up copy of amended paragraph appearing at page 49, lines 4 to 15:

Triethylammonium salt of carboxymethyldextran polyalcohol (200 mg) having an average molecular weight of 337K and a carboxymethylation degree (degree of substitution with carboxymethyl groups per constitutional saccharide residue) of 0.4, which was prepared according to the method described in Example 24 of WO97/46260, was dissolved in DMF (10 ml). The above solution was added with a solution of trifluoroacetic acid salt of H-Gly-Gly-Phe-Gly-NH-(CH₂)₄-CO-DX-8951 (SEQ ID NO. 1) (30 mg) and triethylamine (10 μl) in methanol (4 ml), further added with a solution of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroxyquinoline (200 mg) in methanol (3 ml), and stirred overnight at room temperature with light shielding. The reaction mixture was diluted with 3 M brine, and low molecular weight fractions were removed by an ultrafiltration membrane (50K), and the resulting residue was passed through a 0.22 μm filter and lyophilized to obtain 178 mg of the target compound.

MARKED UP COPY OF AMENDED ABSTRACT

ABTRACT OF THE DISCLOSURE

A [DDS] drug delivery system compound comprising a carboxy(C₁₋₄)alkyldextran polyalcohol modified with a saccharide compound and a residue of drug compound bound to the carboxy(C₁₋₄)alkyldextran polyalcohol, and a method for measuring a [DDS] drug delivery system compound in which a polymer carrier and a residue of drug compound are bound to each other by [means of] a spacer comprising 2 to 8 amino acids linked by peptide bond(s), which comprises [the steps of] treating the [DDS] drug delivery system compound with a peptidase, and measuring the resulting hydrolysate.

MARKED UP COPY OF AMENDED CLAIMS 1-11, 14-20, 22, 23, 26-28, 30 AND 32-38

1. (Amended) A [DDS] drug delivery system compound which comprises a carboxy(C₁₋₄) alkyl dextran polyalcohol modified with [a saccharide compound] galactose or galactosamine and a residue of drug compound bound to the carboxy(C₁₋₄)alkyldextran polyalcohol.
2. (Amended) The [DDS] drug delivery system compound according to claim 1, wherein the carboxy(C₁₋₄)alkyldextran polyalcohol modified with [a saccharide compound] galactose or galactosamine and the residue of drug compound are bound to each other by [means of] a spacer.
3. (Amended) The [DDS] drug delivery system compound according to claim 2, wherein the spacer comprises one amino acid or 2 to 8 amino acids linked by peptide bond(s).
4. (Twice Amended) The [DDS] drug delivery system compound according to claim 1, wherein the carboxy(C₁₋₄) alkyldextran polyalcohol modified with [a saccharide compound] galactose or galactosamine is formed by binding [a saccharide compound] the galactose or galactosamine and a carboxy(C₁₋₄)alkyldextran polyalcohol by [means of] a linker.
5. (Amended) The [DDS] drug delivery system compound according to claim 4, wherein the carboxy(C₁₋₄)alkyldextran polyalcohol modified with [a saccharide compound] galactose or galactosamine has a cluster modification by [saccharide compounds] galactose or galactosamine bound by [means of] a linker.
6. (Amended) A [DDS] drug delivery system compound which is obtainable by binding a residue of a drug compound to a carboxy(C₁₋₄)alkyldextran polyalcohol in which a part of carboxyl groups of the carboxy(C₁₋₄)alkyl moiety are modified with [a saccharide compound] galactose or galactosamine.

7. (Amended) The [DDS] drug delivery system compound according to claim 6, which is obtainable by binding the carboxy(C₁₋₄)alkyldextran polyalcohol and the residue of drug compound by [means of] a spacer.

8. (Twice Amended) The [DDS] drug delivery system compound according to claim 6, which is obtainable by binding the residue of drug compound to the carboxy(C₁₋₄)alkyldextran polyalcohol which is produced by binding the [saccharide compound] galactose or galactosamine or a linker bound with the [saccharide compound] galactose or galactosamine to a part of carboxyl groups of the carboxy(C₁₋₄)alkyl moiety of the carboxy(C₁₋₄)alkyldextran polyalcohol.

9. (Amended) A [DDS] drug delivery system compound which is obtainable by modifying with a [saccharide compound] galactose or galactosamine a carboxy(C₁₋₄)alkyldextran polyalcohol in which a residue of a drug compound is bound to a part of carboxyl groups of the carboxy(C₁₋₄)alkyl moiety by [means of] a spacer.

10. (Amended) The [DDS] drug delivery system compound according to claim 9, which is obtainable by binding the carboxy(C₁₋₄)alkyldextran polyalcohol and the saccharide compound by means of a linker.

11. (Twice Amended) The [DDS] drug delivery system compound according to claim 9, which is obtainable by modifying with a saccharide compound a carboxy(C₁₋₄)alkyldextran polyalcohol produced by binding a residue of drug compound to a part of carboxyl groups of the carboxy (C₁₋₄)alkyl moiety of the carboxy(C₁₋₄)alkyldextran polyalcohol by means of a spacer comprising one amino acid or a spacer comprising 2 to 8 amino acids linked by peptide bond(s).

14. (Amended) The [DDS compounds] drug delivery system compound according to claim [12] 1, wherein substitution degree of galactose or galactosamine [or a derivative thereof],

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or clustered galactose or galactosamine [or derivative thereof] is 0.01-1.0 per saccharide residue of the carboxy(C₁₋₄)alkyldextran polyalcohol.

15. (Twice Amended) The [DDS compounds] drug delivery system compound according to claim 1, wherein the dextran polyalcohol that constitutes the carboxy(C₁₋₄)alkyldextran polyalcohol is a dextran polyalcohol which is obtained by treating dextran under conditions that enable substantially complete polyalcoholization.

16. (Twice Amended) The [DDS] drug delivery system compound according to claim 1, wherein the carboxy(C₁₋₄)alkyldextran polyalcohol is carboxymethyldextran polyalcohol.

17. (Twice Amended) The [DDS] drug delivery system compound according to claim 1, wherein the drug compound is an antineoplastic agent or an anti-inflammatory agent.

18. (Amended) The [DDS] drug delivery system compound according to claim 17, wherein the drug compound is an antineoplastic agent.

19. (Twice Amended) The [DDS] drug delivery system compound according to claim 1, wherein the drug compound is (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione.

20. (Amended) The [DDS] drug delivery system compound according to claim 19, which is a medicament for treating liver cancer.

22. (Amended) A carboxy(C₁₋₄)alkyldextran polyalcohol modified with [a saccharide compound] galactose or galactosamine.

23. (Amended) A polymer carrier comprising a carboxy(C₁₋₄)alkyldextran polyalcohol modified with [a saccharide compound] galactose or galactosamine.

26. (Amended) [The] A method [according to claim 24, which is used] for measurement of content of [the] a residue of a drug compound introduced to [the DDS] a drug delivery system compound in which a polymer carrier and a residue of drug compound are bound to each other by a spacer comprising 2 to 8 amino acids linked by peptide bond(s), which comprises treating the drug delivery system compound with a peptidase, and measuring the resulting hydrolysate.

27. (Twice Amended) The method according to claim [24] 26, wherein the hydrolysate is the drug compound.

28. (Twice Amended) The method according to claim [24] 26, wherein the hydrolysate is a compound comprising the residue of drug compound bound with a part of the spacer.

30. (Twice Amended) The method according to claim [24] 26, wherein the polymer carrier is a polysaccharide derivative having carboxyl groups.

32. (Twice Amended) The method according to claim [24] 26, wherein the drug compound introduced to the [DDS] drug delivery system compound is an antineoplastic agent or an anti-inflammatory agent.

33. (Twice Amended) The method according to claim [24] 26, wherein the spacer is a tetrapeptide represented by -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) from the N-terminal or a tetrapeptide represented by -Gly-Gly-Gly-Phe- (SEQ ID NO. 8) from the N-terminal.

34. (Twice Amended) A method for measuring a drug delivery system compound in which a polymer carrier and a residue of drug compound are bound to each other by a spacer comprising 2 to 8 amino acids linked by peptide bond(s), which comprises treating the drug delivery system compound with a peptidase, and measuring the resulting hydrolysate, and [The method according to claim 24,] wherein the spacer is a group represented by -Gly-Gly-Phe-Gly-HN-Y'-CH₂-O-CO-

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(SEQ ID NO. 1) from the N-terminal or a group represented by -Gly-Gly-Gly-Phe-NH-Y'-CH₂-O-CO- (SEQ ID NO. 8) from the N-terminal wherein Y' represents p-phenylene group.

35. (Twice Amended) A method for measuring a drug delivery system compound in which a polymer carrier and a residue of drug compound are bound to each other by a spacer comprising 2 to 8 amino acids linked by peptide bond(s), which comprises treating the drug delivery system compound with a peptidase comprising [The method according to claim 24, wherein the peptidase is] α -chymotrypsin or papain, and measuring the resulting hydrolysate.

36. (Twice Amended) The method according to claim [24] 26, wherein the drug compound is (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione.

37. (Twice Amended) The method according to claim [24] 34, which is used for measurement of a [DDS] drug delivery system compound in which a carboxy(C₁₋₄)alkyldextran polyalcohol and (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione are bound to each other by [means of] a spacer comprising a tetrapeptide represented by -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) or a tetrapeptide represented by -Gly-Gly-Gly-Phe- (SEQ ID NO. 8) from the N-terminal.

38. (Amended) A method for measuring a drug delivery system compound in which a polymer carrier comprising carboxy(C₁₋₄)alkyldextran polyalcohol and a drug compound comprising (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione are bound to each other by a spacer comprising a tetrapeptide represented by -Gly-Gly-Phe-Gly- (SEQ ID NO. 1) or a tetrapeptide represented by -Gly-Gly-Gly-Phe- (SEQ ID NO. 8) from the N-terminal, which

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comprises treating the drug delivery system compound with a peptidase [The method according to claim 37, wherein] comprising α -chymotrypsin or papain [is used as the peptidase], and measuring (1S,9S)-9-ethyl-5-fluoro-1-glycylamino-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo-[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione [is measured] as the resulting hydrolysate.